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Authors

Kemp, Jeffery
Miller, Lowell

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Oral Vaccination and Immunocontraception of Feral Swine using *Brucella suis* with Multimeric GnRH Protein Expression

Jeffrey M. Kemp and Lowell A. Miller

USDA APHIS Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado

ABSTRACT: As part of the Reproductive Control Methods Project, the USDA National Wildlife Research Center (NWRC) has developed multiple non-lethal contraceptive tools to control population of over-abundant wildlife species. Working in conjunction with Dr. G. P. Talwar of the Talwar Research Institute in India, scientists at NWRC have shown that a recombinant form of GnRH peptide has successfully contracepted swine. Feral swine not only pose a significant agricultural issue due to over-population; they act as a reservoir for *Brucella suis*, a threat to domestic livestock and humans. The *Brucella abortus* RB51 (Rough *Brucella*) vaccine, developed for bovine brucellosis and licensed by the USDA Animal Plant Health Inspection Service, has shown protection for some swine and is also effective against *Brucella suis* infection and other antigens. There is currently no approved vaccine for swine brucellosis (feral or domestic) in the United States. Past studies performed at The Ohio State University show that *Brucella suis* vaccination appears to protect against abortion and colonization in pigs after a virulent challenge.

The Talwar recombinant peptide consists of 5 LHRH peptides interspersed with 4 universally immunogenic “promiscuous” T-cell epitopes of diverse genetic background. By transforming the *Brucella suis* strain with the Talwar recombinant LHRH plasmid, the bacteria will produce both its own host proteins, and the LHRH protein. A systemic immune response should then be generated to the *Brucella suis* proteins being produced, allowing for vaccination to *Brucella suis*. In addition, an immune response would be generated to the LHRH proteins resulting in antibody production and immunocontraception. A broad range of applications can be proposed utilizing the recombinant LHRH, such as other dual vaccines and scale-up production of a low-cost single-injection LHRH vaccine.

KEY WORDS: *Brucella suis*, feral swine, GnRH, immunocontraception, recombinant multimeric GnRH

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INTRODUCTION

Drs. G. P. Talwar and J. C. Gupta of the Talwar Research Foundation in India have engineered a unique recombinant form of GnRH. The genetic sequence encodes for 5 GnRH B-cell epitopes interspersed with 4 immunogenic, promiscuous T-cell epitopes (Gupta et al. 2004). The combination of T-cell and B-cell epitopes is significant, as it causes the production of long-lasting IgG antibodies (Miller et al. 2006). All of the 4 T-cell epitopes are of diverse genetic disease origin, which allows for enhanced immunogenicity (Figure 1). The extracted and purified recombinant multimeric protein (rmGnRH) produced from *Escherichia coli* has a molecular weight between 16 and 18 kDa (Gupta et al. 2004). This protein produces a significant immune response in swine with a single vaccina-

tion (Miller et al. 2006). The recombinant protein’s unique design is highly foreign to the host immune system, and it elicits a sufficiently strong immune response to induce immunocontraception.

Gupta et al. (2004) inserted the multimeric GnRH gene sequence into an *E. coli* expression vector pRSET that is commercially available from Invitrogen (Carlsbad, CA) (Figure 2). This unique plasmid encodes for resistance to ampicillin. The antibiotic sequence applies selection pressure resistance and allows for positive transformant growth in media containing this antibiotic. There-

Recombinant Multimeric GnRH Protein Sequence	
pE-H-W-S-Y-G-L-R-P-G//	-D-I-E-K-K-I-A-K-M-E-K-A-S-S-V-F-N-V-V-N-S//
(GnRH)(1)	(Plasmodium falciparum (Malaria))
-E-H-W-S-Y-G-L-R-P-G//	-Q-Y-I-K-A-N-S-K-F-I-G-I-E-L//
(GnRH)(2)	(Tetanus Toxoid)
-E-H-W-S-Y-G-L-R-P-G//	-A-E-Y-N-V-F-H-N-K-T-F-E-L//
(GnRH)(3)	(Respiratory Syncytial Virus)
-E-H-W-S-Y-G-L-R-P-G//	-L-S-E-I-K-G-V-I-V-H-R-L-E-G-V//
(GnRH)(4)	(Measles Virus)
- E-H-W-S-Y-G-L-R-P-G-NH2	
(GnRH)(5)	

Figure 1. Recombinant multimeric GnRH (rmGnRH) developed by G. P. Talwar (Talwar et al. 2004).

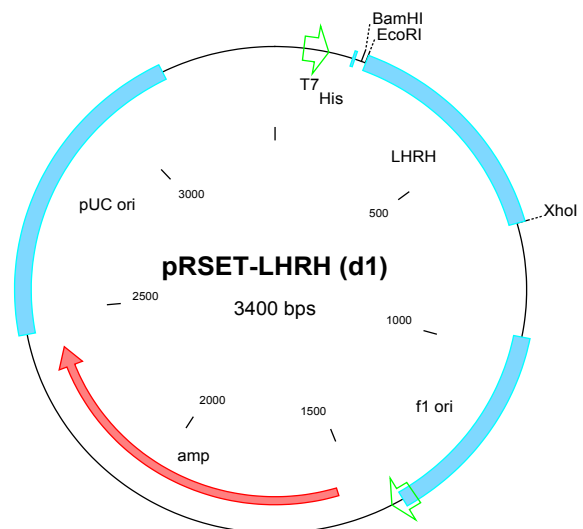


Figure 2. pRSET plasmid and multimeric GnRH sequence insert (Gupta et al. 2004).

Table 1. Comparison of the single and dual injections of the rmGnRH vaccine (Miller et al. 2006).

Treatment	N	Progesterone Positive	Bred	Pregnant
Talwar one injection (750 µg)	5	2/5	3/5	2/5
Talwar two injections (500-500 µg)	5	0/5	0/5	0/5

fore, those cells that do not receive the antibiotic resistance gene from the plasmid do not grow. Those bacteria that do receive the plasmid allow it to begin self-replication, translation of the sequences, and expression of the encoded multimeric GnRH protein. Specific *E. coli* strains, such as BL21(DE3)pLysS, are engineered to over-express recombinant protein for an overall increase in protein yield. Scale-up production of this low-cost recombinant protein produced from an over-expression *E. coli* strain could allow for wide-scale field use and oral delivery of contraceptive vaccine.

Dual Vaccine

As the number of feral swine (*Sus scrofa*) continues to grow, so does the spread of *B. suis*. Not only do the over-abundant feral swine affect crop agriculture, *B. suis* infection in feral swine creates the potential for transmission incidence into food animal populations (Stoffregen et al. 2007). Vaccination to *B. suis* in swine has been attempted using RB51. A positive response was seen, although subsequent, more controlled studies failed to confirm these initial reports (Stoffregen et al. 2007).

Specific bacterial strains, non-infectious to the host organism but sufficiently immunogenic to the immune system, can serve as vectors for heterologous recombinant protein expression and induction of a humeral immune response to the recombinant protein or antigen. Oral vaccination utilizing attenuated bacterial strains is common, with use of RB51 intended to vaccinate cattle and bison against *Brucella abortus* (Vemulapalli et al. 2000). In addition, attenuated *Salmonella* have been utilized in vaccinating mice against tetanus (Fairweather et al. 1990). Certain strains of *Brucella* have recently been studied as potential candidates for a *Brucella* vaccine for domestic and feral swine. For example, strain 353-1 from the National Animal Disease Center in Ames, Iowa is a naturally occurring rough mutant of *Brucella* that shows potential as a viable oral vaccination tool (Stoffregen et al. 2007). The humeral and cell-mediated immune response to 353-1 exposure elicited antibody production that provided systemic protection of swine *B. suis* post challenge.

Because strain 353-1 produces a humeral immune response and allows for protection post challenge, subcloning of the Talwar recombinant sequence from the *E. coli* compatible plasmid, pRSET, into a *Brucella*-compatible plasmid would permit transformation into *B. suis* strain 353-1. Antibodies made to the expressed rmGnRH protein will bind endogenous circulating GnRH. The antibody binding of endogenous GnRH produced by the hypothalamus decreases the normal stimulatory activity of GnRH on the anterior pituitary. This results in decreased LH and FSH secretion leading to decreased fertility.

METHODS

The plasmid construct was successfully transformed into *E. coli* strain BL21(DE3)pLysS, a strain engineered

as a high-protein expression model. The new recombinant bacterial strain confers resistance to ampicillin, confirming a successful transformation. The expressed proteins, mainly localized in the cytosol as insoluble inclusion bodies, are purified by Ni⁺-NTA chromatography (Gupta et al. 2004). Total protein yield will range depending on the bacterial strain.

Contraceptive efficacy utilizing rmGnRH was investigated at Pennsylvania State University (PSU) in 2005 in reproductively mature gilts. The rmGnRH combined with the NWRC adjuvant AdjuVac[™] produced a significant antibody response with a single injection (Miller et al. 2006). Contraception of 3 of 5 gilts was observed: an impressive immunogenic response for a small recombinant (16-18 kDa) protein (Table 1). An additional enhanced response was observed from the treatment group that received a boost injection 30 days after the prime dose. This group exhibited a 100% contraceptive effect (Miller et al. 2006). These results clearly indicate that the rmGnRH protein, in adequate concentrations, is sufficiently immunogenic to cause contraception.

DISCUSSION

The unique design of Talwar's multimeric GnRH gene sequence has allowed for the expression of a highly immunogenic recombinant GnRH (rmGnRH) protein in *E. coli*. These multiple T-cell epitopes provide the enhanced immunogenicity needed to generate a cell-mediated and humeral immune response to the GnRH peptide.

The studies performed at PSU incorporated the rmGnRH protein into an injectable emulsion with AdjuVac[™]. The recombinant protein elicited an impressive anti-GnRH immune antibody response resulting in contraception in 3 of 5 gilts with a single injection, and 5 of 5 gilts with a dual injection. Scale-up production of rmGnRH utilizing high protein yield expression *E. coli* strains will generate increased protein yields at a reasonable cost, thus making oral and large-scale use more feasible.

Inducing a strong systemic immune response with vaccination in feral swine, *B. suis* strain 353-1, a naturally rough mutant, would serve as a vector for the recombinant expression of rmGnRH. Utilized as an oral delivery system, a humeral and cell-mediated immune response to the bacterial strain itself would generate protective antibody production to virulent *B. suis*, as well as immunocontraceptive antibodies to endogenous GnRH, resulting in contraception.

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